

**CLAIMS**

1. A method for producing avian cell lines, wherein it comprises the following steps:

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a) culturing avian cells in a medium containing all the factors allowing their growth and an inactivated feeder layer,

10 b) passage by modifying the culture medium so as to obtain progressive or total withdrawal of said factors, of the serum and/or of the feeder layer,

15 c) establishing adherent or nonadherent cell lines capable of proliferating in a basal medium in the absence of exogenous growth factors, serum and/or inactivated feeder layer.

20 2. The method according to claim 1, wherein the cells derived from the cell lines obtained in step c) are capable of proliferating for at least 50 days, preferably at least 600 days.

25 3. The method according to claim 1, wherein step b) consists in a progressive or total withdrawal of the feeder layer, optionally followed by a progressive withdrawal of the growth factors and/or the serum.

30 4. The method according to claim 1, wherein step b) consists in a progressive or total withdrawal of the growth factors, optionally followed by a progressive withdrawal of the serum.

35 5. The method according to claim 1, wherein step b) consists in a progressive or total withdrawal of the growth factors and/or serum, optionally followed by a withdrawal of the feeder layer.

6. The method according to claim 1, wherein the cells obtained in step c) are subjected to a selection in culture media used for large-scale production so as to obtain clones suitable for the production of vaccine  
5 intended for human or animal therapy.

7. The method according to claim 1, wherein the cells derived from the lines obtained in step c) are avian stem cells.

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8. The method according to claims 7, wherein the cells derived from the lines obtained in step c) are avian embryonic stem cells.

15 9. The method according to claim 7, wherein the cells derived from the lines obtained in step c) are avian somatic stem cells.

20 10. The method according to claim 1, wherein the cells derived from the lines obtained in step c) are adherent stem cells which proliferate in the absence of the inactivated feeder layer.

25 11. The method according to claim 1, wherein the cells derived from the lines obtained in step c) are nonadherent stem cells which proliferate in suspension in a medium free of exogenous growth factors.

30 12. The method according to claim 9, wherein the avian somatic stem cells are nonadherent cells which proliferate in suspension in a medium free of exogenous growth factors.

35 13. The method according to claim 1, wherein the cells derived from the lines obtained in step c) proliferate in a medium free of serum.

14. The method according to claim 9, wherein the avian somatic stem cells are nonadherent cells which proliferate in suspension in a medium free of serum.

5 15. The method according to claim 1, wherein the cells derived from the lines obtained in step c) have at least one of the following characteristics:

- 10       - a high nucleocytoplasmic ratio,
- an endogenous alkaline phosphatase activity,
- an endogenous telomerase activity,
- 15       - a reactivity with specific antibodies selected from the group of antibodies SSEA-1 (TEC01), SSEA-3, and EMA-1.

20 16. The method according to claim 1, wherein the cells derived from the lines obtained in step c) are modified in order to allow a better use *in vitro* such as the extension of the greater life span or growth densities or alternatively of the lower nutrient requirements.

25 17. The method according to claim 1, wherein the cells derived from the lines obtained in step c) are modified in order to produce a substance of interest, in particular a polypeptide of interest, an antibody or an attenuated virus.

30 18. The method according to claim 1, wherein the medium used in step a) comprises at least one factor selected from cytokines, in particular LIF, IL-11, IL-6, IL-6R, CNTF, Oncostatin and other factors such as  
35 SCF, IGF-1 and bFGF.

19. The method according to claim 1, wherein the inactivated feeder layer used in step a) is composed of fibroblast cells including mouse fibroblasts

established as a line, in particular transformed or nontransformed STO cells.

20. The method according to claim 1, wherein the cells  
5 used in step a) are cells obtained by suspending cells obtained from blastodermal disks of fertilized eggs in a culture medium comprising at least one cytokine, b-FGF, and SCF, said cells being inoculated into a layer of feeder cells, incubated, and then collected.

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21. The method according to claim 1, wherein step b) comprises a progressive withdrawal of each growth factor added to the medium in step a), in particular a cytokine, b-FGF, and SCF, comprising a passage in a new  
15 medium free of at least one of said factors and in repeating various successive passages until the medium is free of all of said factors.

22. The method according to claim 21, wherein step b)  
20 additionally comprises the withdrawal of the serum.

23. The method according to claim 21, wherein step b) additionally comprises the withdrawal of the feeder  
layer.

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24. The method according to claim 1, wherein step b) comprises a progressive withdrawal of the serum, comprising successive passages in new media comprising decreased serum concentration and in repeating various  
30 successive passages until the medium is free of serum.

25. The method according to claim 1, wherein step b) comprises the withdrawal of the feeder layer, said withdrawal being either progressive comprising  
35 successive passages in new media comprising decreased feeder cells number and in repeating various successive passages until the medium is free of feeder cells.

26. A cell line and cell derived thereof which can be obtained from the method according to claim 1, wherein it is capable of proliferating for at least 50 days, preferably at least 600 days in a medium free of exogenous growth factor.

27. A cell line and cells derived thereof which can be obtained from the method according to claim 1, wherein it is capable of proliferating for at least 50 days, preferably at least 600 days in a medium depleted of serum and in particular free of serum.

28. A cell line and cells derived thereof which can be obtained from the method according to claim 1, wherein it is capable of proliferating for at least 50 days, preferably at least 600 days in a medium free of feeder layer.

29. A cell line and cells derived thereof which can be obtained from the method according to claim 1, wherein it is capable of proliferating for at least 50 days, preferably at least 600 days in a medium free of exogenous growth factor, depleted of serum or free of serum and/or of feeder layer.

30. A cell line and cells derived thereof which can be obtained from the method according to claim 9, wherein it is capable of proliferating for at least 50 days, preferably at least 600 days in a medium free of exogenous growth factor, depleted of serum or free of serum and/or of feeder layer.

31. The cell line and cells derived thereof according to claims 29 or 30, wherein it is capable of proliferating for at least 50 days, preferably at least 600 days in a basal medium, in particular in a medium such as DMEM, GMEM, HamF12 or McCoy supplemented with various additives such as nonessential amino acids, vitamins and sodium pyruvate.

32. The cell line and cells derived thereof according to claim 26, wherein it is an avian stem cell.

5 33. The cell line and cell derived from such a line according to claim 32, wherein it is an avian embryonic stem cell.

34. The cell line and cells derived thereof according to claim 32, wherein it is an avian somatic stem cell.

35. The cell line and cells derived thereof according to claim 32, wherein it is an adherent stem cell which proliferates in the absence of the inactivated feeder layer.

36. The cell line and cells derived thereof according to claim 32, wherein it is a nonadherent stem cell which proliferates in suspension.

20 37. The cell line and cells derived thereof according to one of claim 32, wherein it has at least one of the following characteristics:

- 25       - a high nucleocytoplasmic ratio,
- an endogenous alkaline phosphatase activity,
- an endogenous telomerase activity,
- 30       - a reactivity with specific antibodies selected from the group of antibodies SSEA-1 (TEC01), SSEA-3, and EMA-1.

35 38. The cell line and cells derived thereof according to one of claims 32, wherein they are genetically modified so as to produce a substance of interest, in particular a polypeptide of interest, an antibody or an attenuated virus.

39. The cell line and cells derived thereof according to claim 38, wherein they support the replication of live or attenuated viruses, in particular the viruses  
5 selected from the group of adenoviruses, hepadnaviruses, herpesviruses, orthomyxoviruses, papovaviruses, paramyxoviruses, picornaviruses, poxviruses, reoviruses and retroviruses.

10 40. The cell line and cells derived thereof according to claim 39, wherein the viruses replicated on these cells belong to the family of orthomyxoviruses, in particular the influenza virus.

15 41. The cell line and cells derived thereof according to claim 39, wherein the replicated viruses belong to the family of paramyxoviruses, in particular the measles, mumps and rubella viruses.

20 42. The cell line and cells derived thereof according to claim 39, wherein the replicated viruses belong to the group of poxviruses such as attenuated vaccinia virus and in particular Avipox virus such as canarypox virus, Fowlpox virus, Juncopox virus, Mynahpox virus,  
25 Pigeonpox virus, Psittacinepox virus, Quailpox virus, Sparrowpox virus, Starlingpox virus and Turkeypox virus.

30 43. A cell line derived from step c) of the method according to one of claims 1, wherein it is a genetically modified avian stem cell capable of growing indefinitely in a basal medium free of exogenous growth factors, depleted of serum and/or free of serum and/or of feeder layer.

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44. The use of the cell line and cells derived thereof according to claim 32 for the production of substances of interest, in particular of proteins of therapeutic interest.

45. The use of the cell line and cells derived thereof according to claim 32, for the replication of live or attenuated viruses, in particular viruses chosen from  
5 the group of adenoviruses, hepadnaviruses, herpesviruses, orthomyxoviruses, papovaviruses, paramyxoviruses, picornaviruses, poxviruses such as vaccinia virus and in particular Avipox virus in particular canarypox virus, Fowlpox virus, Juncopox  
10 virus, Mynahpox virus, Pigeonpox virus, Psittacinepox virus, Quailpox virus, Sparrowpox virus, Starlingpox virus and Turkeypox virus, as well as reoviruses and retroviruses.

15 46. The use of the line according to claim 32, for the production of viruses belonging to the family of orthomyxoviruses, in particular the influenza virus.

20 47. The use of the line according to claim 32 for the production of viruses belonging to the family of paramyxoviruses, in particular the measles, mumps and rubella viruses.

25 48. The use of the line according to claim 39, for supporting the replication of live or attenuated viruses, in particular by introducing the component(s) necessary for accomplishing the complete viral cycle of the virus in the cell, in particular the overexpression of the receptor for the virus at the surface of the  
30 cell.

49. The use according to claim 39, for supporting the replication of live or attenuated viruses, in particular by introducing the component(s) necessary  
35 for accomplishing the complete viral cycle of the virus in the cell, in particular the overexpression of the receptor for the virus at the surface of the cell, said viruses being selected from the group of poxviruses such as vaccinia virus (for example Modified vaccinia



virus Ankara, MVA) and in particular Avipox virus such as canarypox virus, Fowlpox virus, Juncopox virus, Mynahpox virus, Pigeonpox virus, Psittacinepox virus, Quailpox virus, Sparrowpox virus, Starlingpox virus and  
5 Turkeypox virus.

50. The use according to claim 39 to produce live or attenuated vaccine comprising culturing the adherent or non adherent cell lines established in step c)  
10 according to the process described above, inoculating said cells with viral particles and culturing said cells in a basal medium as mentioned above until cell lysis occurs and newly produced viral particles are released in said medium.

15 51. The use according to claim 39 to produce live or attenuated, native or recombinant, vaccine selected from the group consisting of the family of adenoviruses (such as Human Adenovirus C, Fowl Adenovirus A, Ovine  
20 Adenovirus D, Turkey Adenovirus B), circoviridae (such as Chicken Anemia Virus, CAV), coronaviruses, such as avian infectious bronchitis virus (IBV), flaviviruses (such as Yellow fever virus and hepatitis C virus), hepadnaviruses (such as Hepatitis B virus and  
25 Avihepadnaviruses such as Duck hepatitis B virus); herpesviruses (such as Gallid herpesvirus, HSV (Herpes simplex virus) and Human herpesvirus 1, 3 and 5), orthomyxoviruses (such as the influenza virus: Influenzavirus A, Influenzavirus B and Influenza-  
30 virus C), papovaviruses (such as polyomavirus and more particularly Simian virus 40), paramyxoviruses (such as measles, mumps and rubella viruses and such as respiroviruses and pneumoviruses such as human respiratory syncytial virus and Metapneumovirus such as  
35 Avian pneumovirus), picornaviruses (such as polio virus, hepatitis A virus, and such as Encephalomyocarditis virus and foot-and-mouth disease virus), poxviruses (such as fowlpox virus and avipox viruses including Canarypox viruses, Juncopox viruses,

Mynahpox viruses, Pigeonpox viruses, Psittacinepox viruses, Quailpox viruses, Sparrowpox viruses, Starlingpox viruses, Turkeypox viruses), orthopoxvirus such as vaccinia virus, MVA, and reoviruses (such as  
5 rotaviruses), retroviruses (such as ALV, avian leukosis virus, Gammaretroviruses such as Murine leukemia virus, Lentiviruses such as Human immunodeficiency virus 1 and 2) and Togaviridae such as Rubivirus, in particular Rubella virus.

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52. The use according claim 45 to produce a vaccine against smallpox.

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53. The use according claim 45 to produce a recombinant vaccine against cancer.